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Minireview

Bridging the gap: From protein misfolding to protein misfolding diseases

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ABSTRACT

Protein misfolding and aggregation are pathognomic for a number of the most common age-related degenerative diseases. Great progress has been made in studying protein aggregation in the test tube and also in replicating protein aggregation in vertebrate animal models of these diseases. However, we argue here that the development and effective integration of emerging techniques such as the methods of nanoscience and the use of invertebrate models are now providing powerful new opportunities to advance our current understanding of the fundamental origins of these disorders.

1. Introduction

The ability of polypeptide chains in vivo to fold correctly into their native states with sufficient frequency for them to be able to execute their functions in a living organism is one of the most fundamental and remarkable phenomena in biology. Whilst proteins have evolved to contain all the information required to adopt their correct architecture within their own amino acid sequences, they are assisted in achieving this fold in vivo by molecular chaperones that accelerate and guide this process [1]. Mechanisms such as the unfolded protein response have also evolved in the cell for sensing and responding to an excess of misfolded proteins and for targeting them to be degraded by proteolytic systems such as the proteosome [2]. However, despite these multiple regulatory systems and the intrinsic ability of evolved protein sequences to avoid aggregation, protein misfolding and aggregation nevertheless occur, particularly as organisms age, and can cause devastating diseases [3].

Since the original description of protein deposits in human tissue by Virchow in 1854 (reviewed in [4]), that he termed amyloid for their apparently tinctorial starch-like properties, a great deal has been discovered about the origins of such protein deposits, the processes by which they are formed and in particular their association with both the physiology and pathology of organisms ranging from fungi to humans [5]. As the proteins of which amyloid deposits are primarily composed in both neurological conditions such as Alzheimer's Disease (AD), and non-neuropathic conditions such as Type II diabetes have been identified, researchers have predominantly adopted two distinct approaches to probe the origins and consequences of the misfolding and aggregation of these proteins; the investigation of protein misfolding in vitro using biophysical techniques and the generation of transgenic mammalian models replicating the aggregation of proteins associated with disease in vivo.

In this review we argue that these two approaches have, when carried out independently, yielded a large number of important insights into the physics and the pathology of protein misfolding. However, we also discuss how, using new models and methods, we can bridge the gap between them, enabling us to progress towards translating these insights into meaningful improvements in the diagnosis and treatment of the many devastating diseases associated with protein misfolding.

2. Of mice and men with test tubes

The study of protein misfolding and its association with disease has been transformed by identification of the genes whose protein products are responsible for these diseases. This information has not only enabled the generation of transgenic models, mainly in mice, of protein misfolding disorders but also has allowed the production of these proteins recombinantly and hence the study of

Abbreviations: SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; AD, Alzheimer's Disease; Aβ, Amyloid Beta; APP, Amyloid Precursor Protein; AFM, Atomic Force Microscopy; TCCD, Two Colour Coincidence Detection; QCM, Quartz Crystal Microbalance; SEC, Size Exclusion Chromatography; FRAP, Fluorescence Recovery After Photobleaching; sHsp, small Heat shock protein

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their properties in vitro. Perhaps the most surprising observations to come out of this early period of research were the sheer diversity of the types of proteins that misfold and aggregate into amyloid structures (reviewed in [5]) and that the range of proteins able to convert into the amyloid state is not restricted to those that have been identified in disease pathology [6–9]. Subsequently the formation of amyloid fibrils has also been shown not only to be associated with pathology but also with functionality in organisms ranging from *Escherichia coli* to humans [10,11].

The challenge that arose from these observations was to understand why certain proteins are found in amyloid deposits in vivo under physiological or pathological conditions, whereas others, whilst intrinsically capable of aggregating into these amyloid states do so only under non-native conditions. A number of classical biophysical studies of the folding and misfolding of proteins associated with amyloidosis, such as lysozyme and transthyretin (TTR) [12,13], along with studies of 'model' proteins such as acylphosphatase [8] have revealed that aggregation of such folded proteins at detectable rates requires that they become at least partially destabilised and 'expose' aggregation prone sequences that are capable of self-interaction. In some cases the intrinsic fluctuations in the native structures provides a mechanism for facilitating such exposure, but in other more stable proteins a partial unfolding transition is necessary [14].

A number of amyloid-related diseases are associated with mutations or abnormal cleavage of the amyloidogenic proteins and it is clear that these factors act either to destabilise the native state of the protein itself or to increase the aggregation propensity of the exposed regions in its polypeptide sequence. Further mutagenic studies based on these principles have now brought us to the stage at which we are able to rationalise, with a high degree of confidence, the propensity of different proteins to aggregate into amyloid fibrils from a knowledge of their amino acid sequences and the protection afforded to different regions of a sequence by folding [15–17].

In parallel with these biochemical attempts to understand the physicochemical basis of protein misfolding, biomedical scientists have constructed a wide range of transgenic models that attempt to capture the major features of both neurological conditions and systemic amyloidoses. Much attention has been directed at Alzheimer's Disease as it is the most common neurodegenerative disease and is becoming increasingly prevalent as our population ages. It is associated with both classical extracellular amyloid deposits composed of Amyloid β peptides (aggregation prone cleavage fragments of the Amyloid Precursor Protein (APP)), and also intracellular inclusions in the form of neurofibrillary tangles of the microtubule associated protein tau (reviewed in [18]). There is now a wide range of transgenic models of AD based on the overexpression of various combinations of mutants of either APP, presenilins that form part of the gamma secretase complex which is responsible for Aβ cleavage from APP, or the tau protein itself (reviewed in [19]; see www.alzforum.org for an up to date list). Many of these transgenic animal models successfully reproduce the well know Aβ plaques, and the neurofibrillary tangles of tau that are found in AD, and so are widely used both in basic research and in the quest for new treatments.

One of the most important insights that has been gained from models such as those discussed in the previous paragraph is, however, the uncertain nature of the relationship between these deposits and the pathogenesis of the diseases with which they are associated. The observation that the Aβ plaque burden, for example, can be a poor indicator of cognitive decline in these models [20,21], is consistent with neuropathological studies in humans [22–24]. Moreover, recent clinical trial results suggest that removing existing Aβ plaques from the brain may not reverse or even halt cognitive decline [25]. The most commonly postulated, and best supported explanations of these observations are now that, contrary to the classical fibrillocentric view of amyloid-related diseases, it is the precursors to amyloid formation, so called prefibrillar aggregates or oligomers that are likely to be primarily responsible for cellular damage in many cases [26]. Furthermore it has been proposed that these prefibrillar aggregates may initiate a number of cellular signals and responses that may in time become self-perpetuating and independent of the initial misfolded protein insult [27].

Therefore, as the field moves away from the view that this type of protein misfolding diseases are caused by relatively inert fibrillar deposits and towards a view in which these diseases are primarily (but not exclusively) caused by relatively soluble, dynamic and heterogeneous precursor aggregates that may interact with multiple cellular components, our approaches to tackling these diseases must adapt accordingly. We require new biophysical tools to understand the structure and dynamic behaviour of the various types of fibrillar and prefibrillar aggregates and new models of disease in which we are able to study the consequences of their formation with greater spatial, temporal and structural resolution.

3. Advances on the biophysical front – new tools for probing oligomer and fibril formation in vitro

As discussed above, one of the key challenges facing the protein misfolding field in adapting to the prevailing oligomeric view of diseases such as AD is to be able to describe accurately the structure and dynamics of the heterogeneous population of species formed during amyloid aggregation. This step is essential if we are to determine which oligomeric species of aggregates are responsible for causing disease and to identify ways of either preventing their formation, enhancing their removal or blocking their effects.

Amyloid fibrils themselves were distinguished from other fibrous structures very early after their discovery by their tinctorial properties [28]. Consequently dyes such as Congo Red and Thioflavin T that bind to amyloid fibrils have been widely used in monitoring the kinetics of amyloid aggregation [29]. These methods have also been complemented by the measurement of the acquisition of the β-sheet structure that is typical of amyloid fibrils using spectroscopic techniques such as Circular Dichroism and Fourier Transform Infrared Spectroscopy [29]. These methods enable a great deal of information to be garnered on the kinetics of protein aggregation, and enable us to identify, for example, other molecules that can modulate this process. Nevertheless, these methods are limited by the resolution with which they can describe the dynamic and heterogeneous processes that underlie protein aggregation, the bulk nature of these measurements, the lack of specificity of the dyes in binding and the fact that β-sheet structure is common to different conformations of aggregates. Consequently it is difficult to extract information specifically about oligomer and fibril behaviour from these measurements.

For this reason researchers have sought methods of separately and accurately probing oligomer formation and fibril growth and dynamics in vitro. These methods take their inspiration from the techniques of nanoscience and of single molecule detection. The key problem in characterising the oligomerisation process that precedes fibril formation is that this process is very heterogeneous. Oligomerisation is thought to be initiated by the coalescence of unfolded, aggregation prone segments of polypeptide chains [30,31]. This process gives rise to oligomeric species with a distribution of size and conformational properties. During the course of oligomer formation, the kinetically driven initial state reorganises to give more thermodynamically stable states, the most highly organised

of which can be visualised using Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM) [32,33], and can even be detected, albeit at quite low temporal and size resolution, using techniques such as Size Exclusion Chromatography (SEC) [34].

The development of Two Colour Coincidence Detection (TCCD), can however allow us to overcome these limitations of resolution and sample heterogeneity. This method has been used successfully to characterise with single molecule resolution a heterogeneous population of oligomers, formed by an SH3 domain and to monitor how it evolves over time [35]. In TCCD a mixture of protein monomers, each labelled with one of two fluorophores that emit light at different wavelengths, is allowed to aggregate and the resulting population of aggregates, is diluted to a concentration at which single particles, oligomers or monomers, can be observed and distinguished using dual excitation in a confocal laser microscope. The coincident detection of both emitted wavelengths indicates the presence of aggregates consisting of more than one molecule and the intensity of the light emitted and frequency of such coincident bursts can then be used to determine the size of the aggregates present, their abundance within the population and even their stability (Fig. 1). The application of this technique to proteins known to be involved in protein misfolding diseases has the potential to unravel much of the confusion that still surrounds the nature of the oligomerisation of key proteins such as Aβ, tau and α synuclein.

Whilst the recent focus of much research into aggregation disorders has been on understanding protein oligomerisation, it is clear that the process of conversion into fibrils remains crucial to our understanding of misfolding diseases. This is not only because the deposition of large quantities of fibrils are very likely to cause significant damage to tissues such as the brain, and indeed are likely to be the main source of damage in some systemic amyloidoses [36], but also as there is now increasing evidence that amyloid fibrils are themselves more dynamic than previously anticipated and may therefore provide an ongoing source of more toxic fibrillar precursors in vivo [37–39].

Studies of amyloid formation in bulk solution are, however, as discussed above, complicated by the plethora of species that can be present during a typical aggregation reaction. We are now, however, able to measure fibril growth separately from any other competing processes, such as oligomerisation, using a technique 'borrowed' from nanoscience, the Quartz Crystal Microbalance (QCM) (Fig. 2) [40,41]. A QCM is essentially a mass sensor that is able to detect the elongation of fibrils attached to a layer of gold deposited on to the surface of a quartz crystal; detection of fibril growth is possible by measuring the alterations in the resonant frequency of the quartz crystal that occur as the mass of the fibrils attached to the surface is increased. This method allows not only the direct determination of growth rates of fibrils but, by scanning the surface using AFM, it allows the number of fibrils present and their morphologies to be determined. We have shown that this method can be used not only to measure the elongation rate with remarkable accuracy but also to describe unambiguously the inhibition of fibril elongation by chaperones [42], a phenomenon that is difficult to detect in bulk solution where chaperones are also known to interact with a variety of species.

4. Advances on the biological front – improving structural and temporal resolution of protein aggregation in vivo

Whilst the new biophysical and nanoscience techniques described above, along with others being developed, will doubtless allow us to characterise protein aggregation in the future with much higher accuracy, and greater structural and temporal resolution, the problem remains of how to translate these advances into an improved understanding of the mechanism of the aggregation of proteins in vivo and its relationship with the pathogenesis of disease in humans. There is currently something of a chasm between our knowledge of the types of protein aggregates that can form in vitro, where the behaviour of even single particles can be measured on timescales of seconds or less, and those species formed in vivo where large numbers of aggregates are typically pooled from tissue samples collected at intervals of days to months.

The accurate identification of specific conformations of aggregates in vivo and their correlation with cognitive dysfunction, or other neurological disease-associated phenotypes in humans or indeed animal models relies on their extraction from the brain without perturbing their composition. Thus, whilst approaches such as sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and Size Exclusion Chromatography allow the determination of the size of aggregated species that are stable and well populated, they cannot detect potentially important species that may not possess these characteristics.

One striking recent example of what can be achieved when new methods are used to overcome these limitations is the use of Two-Photon Confocal Microscopy and fluorescent amyloid binding dyes to monitor the process of Aβ plaque formation in a transgenic mouse model of Alzheimer's Disease [37]. In this study, daily imaging of the brain in live animals revealed that Aβ plaques appear on unexpectedly short time scales (as little as 24 h) and are remarkably stable in size after that time. Studies such as these, which fundamentally alter view of protein aggregation kinetics in vivo, show

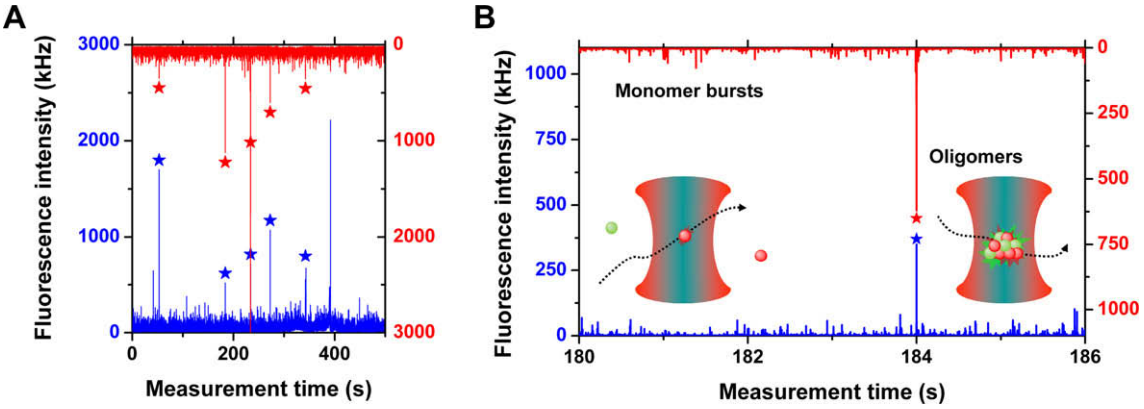

Fig. 1. Two Colour Coincidence Detection of oligomeric aggregates. (A) Detection of oligomer events in PI3–SH3 aggregation. The coincident fluorescent bursts on both channels show the presence of oligomers (marked as asterisks). (B) Expansion of fluorescence bursts in (A). Comparison of the intensity of bursts from monomers and oligomers: the monomer fluorescent bursts are not coincident and are much less intense than those arising from oligomer fluorescence. Figure taken from Orte, Birkett, Clark, et al. PNAS 105 (38), 14424–14429.

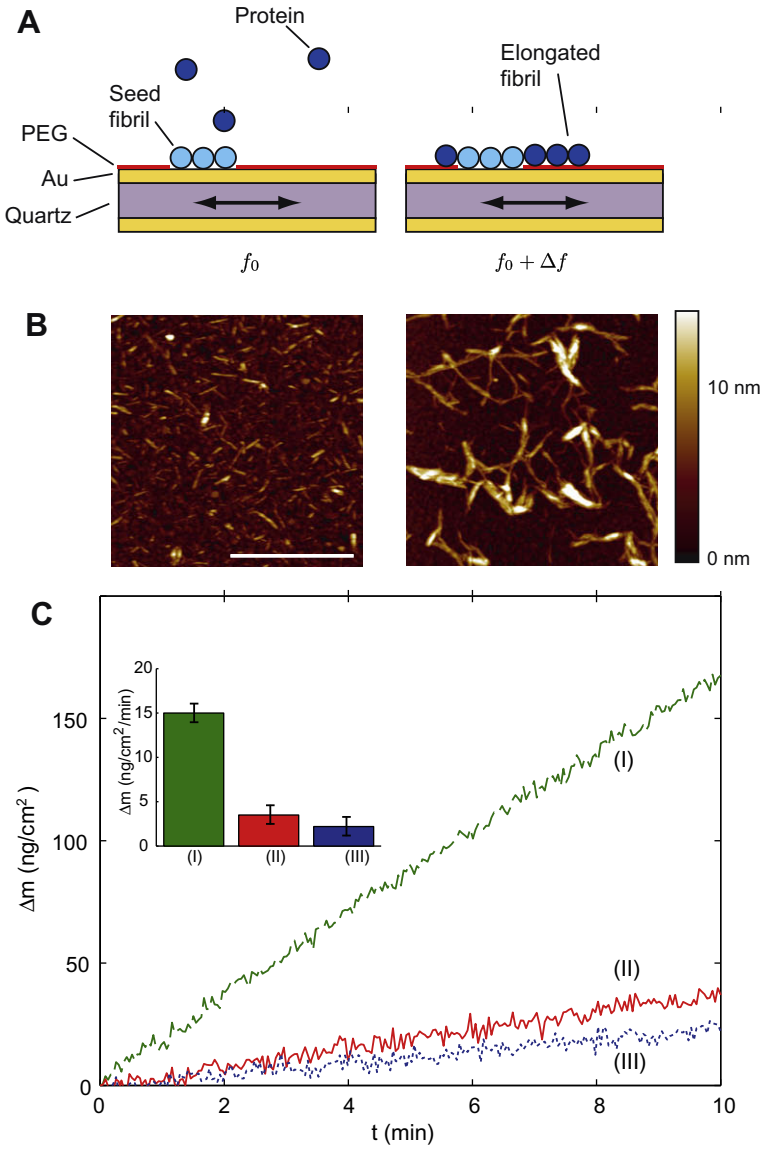

Fig. 2. Quartz Crystal Microbalance measurement of fibril elongation. (A) Illustration of the principle of fibril growth rate determination by the QCM method. Seed fibrils are attached to the gold surface that is then passivated using polyethylene glycol (PEG) and a solution of protein molecules is allowed to flow across it. As monomers attach to the fibril the frequency of the quartz crystal oscillation changes in proportion to the increase in fibril mass. (B) AFM images of the QCM chip before fibril elongation where only short seed fibrils are present (left) and after fibril elongation where longer and larger fibrils are now visible (right). (C) The effect of a small Heat Shock Protein (sHsp) on fibril growth is demonstrated by incubating an insulin fibril seeded QCM chip first with an insulin solution in buffer (I, green dashed line) and then to a mixture of 0.5:1 molar ratio of sHsp to insulin (II, red solid line), which inhibits fibril growth. The sHsp binds to fibril elongation sites and significantly reduces the rate of further fibril elongation even after the sHsp solution is replaced with a solution of insulin in buffer (III, blue dashed line). Figure adapted from Knowles et al. PNAS 104 (24), 10016–10021.

the importance not only of obtaining information with a higher temporal resolution but also of being able to characterise specific conformations of aggregates in their natural environments.

Further advances towards achieving in vivo the level of structural resolution of protein aggregates that can be achieved in vitro are now being made through the development of conformation specific binding proteins. The revolutionary observation was made in 2003 that antibodies can be raised that recognised particular conformational features associated with specific types of aggregates and not just sequence similarity [43]. This development made it clear that such tools could, in principle, allow researchers to overcome the limitations of traditional methods of extracting and analysing protein aggregates. A range of oligomer and fibril specific antibodies has now been produced and has been shown to be a valuable tool for correlating aggregate conformation with disease phenotypes in vivo. Following on from the first generation of such antibodies, a new generation of engineered single-chain binding proteins, e.g. camelid antibody domains, affibodies and intrabodies, have now been developed by several groups to detect specific misfolded conformations of proteins and aggregates [44,45]. Such single-chain binding proteins offer potential advantages over conventional antibodies as they are easily produced, engineered and characterised in vitro and can be selected from libraries using well-characterised conformationally homogeneous antigens. These various different types of conformation-specific binding proteins have the advantage that they can be applied in vivo (through injection or direct expression) to bind to and block the effects of the specific types of aggregates to which they bind, allowing the effects of perturbing particular steps in the aggregation pathway to be determined directly in living organisms giving both high temporal and structural resolution [46,47].

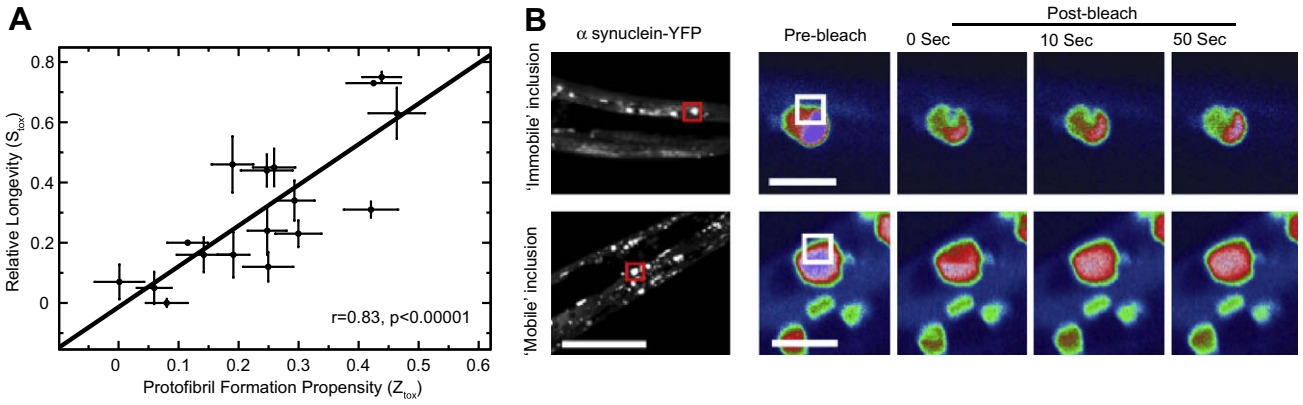

Fig. 3. Quantitative in vivo analysis of protein aggregation and neurotoxicity in *D. melanogaster* and *C. elegans*. (A) Graph showing the strong correlation (83%) between the predicted propensity of 17 mutant Aβ peptides to form protofibrils (Z_{tox}) and their neurotoxicity (S_{tox}) in a *Drosophila* model of neurodegeneration. (B) Assessment of the solubility of α synuclein-YFP aggregates *in situ* in *C. elegans*. Fluorescence Recovery After Photobleaching (FRAP) shows that two types of α synuclein inclusions are present in the worm; 'immobile' inclusions contain poorly diffusible, presumably less soluble, material, whereas 'mobile' inclusions contain diffusible, relatively soluble material. Figure adapted from Luheshi et al. (2007) PLoS Biol. 5 (11), e290 and Van Ham et al. (2008) PLoS Genet. 4 (3), e1000027.

5. New model organisms – small is beautiful

The depth of our understanding of the mechanisms of protein misfolding and aggregation in vitro depends on our ability to make very large numbers of measurements rapidly and with increasingly high structural resolution [48]. If we are to discover whether the same principles that have been found to govern protein aggregation in vitro, also govern protein aggregation in vivo we must start to embrace the use of model organisms that afford us the same opportunities as those available to biophysicists by test tubes i.e. transparency, abundance and simplicity. This challenge has been taken up by a number of groups working on protein misfolding who now make extensive use of three classical model organisms *Saccharomyces cerevisiae*, *Caenorhabditis elegans* and *Drosophila melanogaster* which possess between them most of the features required to act as good 'living test tubes' (reviewed in [48]).

We have shown, for example, that *Drosophila* make an ideal system in which to perform relatively high-throughput mutagenic analysis of the aggregation of the Aβ peptide and its relationship to neuronal dysfunction. The advantages of short lifespans and abundance have allowed us to use *Drosophila* to determine that the neurodegeneration caused by expressing different mutant Aβ peptides in the brain varies dramatically, but correlates strongly with their propensities to aggregate into prefibrillar species in vitro (Fig. 3) [49]. Indeed *Drosophila* are particularly well suited to studying neurodegeneration as they have a sufficiently complex nervous systems to allow measurements not only of longevity (a surrogate for neurodegeneration) but also of locomotor behaviour and even learning and memory [49,50]. The compound eye of *Drosophila* also provides an externally visible population of neurons whose degeneration can be easily monitored and which can be useful in, for example, performing high-throughput genetic screens.

Whilst such correlative analysis of aggregation and neurodegeneration is very valuable, other physiologically less complex model organisms, in particular *C. elegans*, offer better opportunities to visualise protein aggregation directly in the living organism and to relate it to behavioural abnormalities and degeneration. Here the transparency of *C. elegans* is key, allowing aggregation-prone polypeptide sequences fused to fluorescent proteins to be readily visualised by fluorescence microscopy as they aggregate [51]. Differences in the aggregation states of the proteins can even be determined in situ by examining the mobility of the fluorescent proteins using techniques such as Fluorescence Recovery After Photobleaching (FRAP) (Fig. 3) [52]. Such studies represent important advances as they allow us to monitor protein aggregation as it occurs in its normal physiological setting whilst simultaneously assessing the behaviour of an organism for signs of disease-like phenotypes. Furthermore the easy visualisation of aggregates combined with the available genetic resources for manipulating *C. elegans* has facilitated a number of genetic screens for modifiers of the protein misfolding and aggregation processes [52].

6. Perspectives

In this review we have highlighted a selection of the new tools and new methods now being employed to study protein misfolding in vitro and in vivo. The key, we believe, to making greater progress in our goal of understanding the causes and consequences of protein misfolding lies in ever closer integration of the different approaches described here, with a particular emphasis on relating in vivo and in vitro studies. The increasing abundance of tools with which to monitor the process of aggregation and the behaviour of aggregates in vivo, in combination with models of protein misfolding and its effects that are sufficiently complex to be relevant to disease and yet simple enough to be amenable to high-throughput and increasingly sophisticated biophysical and behavioural analysis will, we believe, provide us with unprecedented insights into the pathogenesis of protein misfolding disorders such as Alzheimer's and Parkinson's Diseases. Furthermore these new approaches also have the potential to elucidate the underlying intrinsic and extrinsic factors that regulate the behaviour of proteins as they strive to fold and function within cells to enable living organisms to thrive and compete in the process of evolution.

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References

[1] Bukau, B., Weissman, J. and Horwich, A. (2006) Molecular chaperones and protein quality control. Cell 125, 443–451.
[2] Ron, D. and Walter, P. (2007) Signal integration in the endoplasmic reticulum unfolded protein response. Nat. Rev. Mol. Cell Biol. 8, 519–529.
[3] Dobson, C.M. (2003) Protein folding and misfolding. Nature 426, 884–890.
[4] Sipe, J.D. and Cohen, A.S. (2000) Review: history of the amyloid fibril. J. Struct. Biol. 130, 88–98.

[5] Chiti, F. and Dobson, C.M. (2006) Protein misfolding, functional amyloid, and human disease. Annu. Rev. Biochem. 75, 333–366.
[6] Dobson, C.M. (1999) Protein misfolding, evolution and disease. Trends Biochem. Sci. 24, 329–332.
[7] Fandrich, M., Fletcher, M.A. and Dobson, C.M. (2001) Amyloid fibrils from muscle myoglobin. Nature 410, 165–166.
[8] Chiti, F., Webster, P., Taddei, N., Clark, A., Stefani, M., Ramponi, G. and Dobson, C.M. (1999) Designing conditions for in vitro formation of amyloid protofilaments and fibrils. Proc. Natl. Acad. Sci. USA 96, 3590–3594.
[9] Guijarro, J.I., Sunde, M., Jones, J.A., Campbell, I.D. and Dobson, C.M. (1998) Amyloid fibril formation by an SH3 domain. Proc. Natl. Acad. Sci. USA 95, 4224–4228.
[10] Chapman, M.R., Robinson, L.S., Pinkner, J.S., Roth, R., Heuser, J., Hammar, M., Normark, S. and Hultgren, S.J. (2002) Role of *Escherichia coli* curli operons in directing amyloid fiber formation. Science 295, 851–855.
[11] Fowler, D.M., Koulov, A.V., Alory-Jost, C., Marks, M.S., Balch, W.E. and Kelly, J.W. (2006) Functional amyloid formation within mammalian tissue. PLoS Biol. 4, e6.
[12] Booth, D.R. et al. (1997) Instability, unfolding and aggregation of human lysozyme variants underlying amyloid fibrillogenesis. Nature 385, 787–793.
[13] Lai, Z., Colon, W. and Kelly, J.W. (1996) The acid-mediated denaturation pathway of transthyretin yields a conformational intermediate that can self-assemble into amyloid. Biochemistry 35, 6470–6482.
[14] Chiti, F. and Dobson, C.M. (2009) Amyloid formation by globular proteins under native conditions. Nat. Chem. Biol. 5, 15–22.
[15] Tartaglia, G.G., Pawar, A.P., Campioni, S., Dobson, C.M., Chiti, F. and Vendruscolo, M. (2008) Prediction of aggregation-prone regions in structured proteins. J. Mol. Biol. 380, 425–436.
[16] DuBay, K.F., Pawar, A.P., Chiti, F., Zurdo, J., Dobson, C.M. and Vendruscolo, M. (2004) Prediction of the absolute aggregation rates of amyloidogenic polypeptide chains. J. Mol. Biol. 341, 1317–1326.
[17] Chiti, F., Stefani, M., Taddei, N., Ramponi, G. and Dobson, C.M. (2003) Rationalization of the effects of mutations on peptide and protein aggregation rates. Nature 424, 805–808.
[18] Selkoe, D.J. and Podlisny, M.B. (2002) Deciphering the genetic basis of Alzheimer's disease. Annu. Rev. Genom. Human Genet. 3, 67–99.
[19] Gotz, J. and Ittner, L.M. (2008) Animal models of Alzheimer's disease and frontotemporal dementia. Nat. Rev. Neurosci. 9, 532–544.
[20] Oddo, S. et al. (2003) Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. Neuron 39, 409–421.
[21] Westerman, M.A. et al. (2002) The relationship between Abeta and memory in the Tg2576 mouse model of Alzheimer's disease. J. Neurosci. 22, 1858–1867.
[22] Lue, L.F. et al. (1999) Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. Am. J. Pathol. 155, 853–862.
[23] Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS) (2001) Pathological correlates of late-onset dementia in a multicentre, community-based population in England and Wales. The Lancet 357, 169–175.
[24] Reiman, E.M. et al. (2009) Fibrillar amyloid-Œ⩽burden in cognitively normal people at 3 levels of genetic risk for Alzheimer's disease. Proc. Natl. Acad. Sci. 106, 6820–6825.
[25] Holmes, C. et al. (2008) Long-term effects of A[beta]42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial. The Lancet 372, 216–223.
[26] Haass, C. and Selkoe, D.J. (2007) Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid [beta]-peptide. Nat. Rev. Mol. Cell. Biol. 8, 101.
[27] St George-Hyslop, P.H. and Morris, J.C. (2008) Will anti-amyloid therapies work for Alzheimer's disease? The Lancet 372, 180–182.
[28] Westermark, P. et al. (2005) Amyloid: toward terminology clarification. Report from the Nomenclature Committee of the International Society of Amyloidosis. Amyloid 12, 1–4.
[29] Nilsson, M.R. (2004) Techniques to study amyloid fibril formation in vitro. Methods 34, 151–160.
[30] Serio, T.R., Cashikar, A.G., Kowal, A.S., Sawicki, G.J., Moslehi, J.J., Serpell, L., Arnsdorf, M.F. and Lindquist, S.L. (2000) Nucleated conformational conversion and the replication of conformational information by a prion determinant. Science 289, 1317–1321.
[31] Cheon, M., Chang, I., Mohanty, S., Luheshi, L.M., Dobson, C.M., Vendruscolo, M. and Favrin, G. (2007) Structural reorganisation and potential toxicity of oligomeric species formed during the assembly of amyloid fibrils. PLoS Comput. Biol. 3, 1727–1738.
[32] Lashuel, H.A., Hartley, D.M., Petre, B.M., Wall, J.S., Simon, M.N., Walz, T. and Lansbury Jr., P.T. (2003) Mixtures of wild-type and a pathogenic (E22G) form of Abeta40 in vitro accumulate protofibrils, including amyloid pores. J. Mol. Biol. 332, 795–808.
[33] Mastrangelo, I.A., Ahmed, M., Sato, T., Liu, W., Wang, C., Hough, P. and Smith, S.O. (2006) High-resolution atomic force microscopy of soluble A[beta]42 oligomers. J. Mol. Biol. 358, 106–119.
[34] Paivio, A., Jarvet, J., Graslund, A., Lannfelt, L. and Westlind-Danielsson, A. (2004) Unique physicochemical profile of beta-amyloid peptide variant Abeta1-40E22G protofibrils: conceivable neuropathogen in arctic mutant carriers. J. Mol. Biol. 339, 145–159.
[35] Orte, A., Birkett, N.R., Clarke, R.W., Devlin, G.L., Dobson, C.M. and Klenerman, D. (2008) Direct characterization of amyloidogenic oligomers by single-molecule fluorescence. Proc. Natl. Acad. Sci. USA 105, 14424–14429.
[36] Pepys, M.B. (2006) Amyloidosis. Annu. Rev. Med. 57, 223–241.
[37] Meyer-Luehmann, M. et al. (2008) Rapid appearance and local toxicity of amyloid-beta plaques in a mouse model of Alzheimer's disease. Nature 451, 720–724.
[38] Spires-Jones, T.L., Meyer-Luehmann, M., Osetek, J.D., Jones, P.B., Stern, E.A., Bacskai, B.J. and Hyman, B.T. (2007) Impaired spine stability underlies plaque-related spine loss in an Alzheimer's disease mouse model. Am. J. Pathol. 171, 1304–1311.
[39] Carulla, N. et al. (2005) Molecular recycling within amyloid fibrils. Nature. 436, 554–558.
[40] Hiroaki Okuno, K.M.T.J.H.I.S.C. (2006) Convenient method for monitoring Abeta aggregation by quartz-crystal microbalance. Chem. Biol. Drug Des. 68, 273–275.
[41] White, D.A., Buell, A.K., Dobson, C.M., Welland, M.E. and Knowles, T.P.J. (2009). Biosensor-based label-free assays of amyloid growth, FEBS Lett., this issue, doi 10.1016/j.febslet.2009.06.008.
[42] Knowles, T.P., Shu, W., Devlin, G.L., Meehan, S., Auer, S., Dobson, C.M. and Welland, M.E. (2007) Kinetics and thermodynamics of amyloid formation from direct measurements of fluctuations in fibril mass. Proc. Natl. Acad. Sci. USA 104, 10016–10021.
[43] Kayed, R., Head, E., Thompson, J.L., McIntire, T.M., Milton, S.C., Cotman, C.W. and Glabe, C.G. (2003) Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis. Science 300, 486–489.
[44] Hoyer, W., Gronwall, C., Jonsson, A., Stahl, S. and Hard, T. (2008) Stabilization of a beta-hairpin in monomeric Alzheimer's amyloid-beta peptide inhibits amyloid formation. Proc. Natl. Acad. Sci. USA 105, 5099–5104.
[45] Habicht, G. et al. (2007) Directed selection of a conformational antibody domain that prevents mature amyloid fibril formation by stabilizing Abeta protofibrils. Proc. Natl. Acad. Sci. USA 104, 19232–19237.
[46] Oddo, S., Caccamo, A., Tran, L., Lambert, M.P., Glabe, C.G., Klein, W.L. and LaFerla, F.M. (2006) Temporal profile of amyloid-beta (Abeta) oligomerization in an in vivo model of Alzheimer disease. A link between Abeta and tau pathology. J. Biol. Chem. 281, 1599–1604.
[47] Chartier, A., Raz, V., Sterrenburg, E., Verrips, C.T., van der Maarel, S.M. and Simonelig, M. (2009) Prevention of oculopharyngeal muscular dystrophy by muscular expression of Llama single-chain intrabodies in vivo. Human Mol. Genet. 18, 1849–1859.
[48] Luheshi, L.M., Crowther, D.C. and Dobson, C.M. (2008) Protein misfolding and disease: from the test tube to the organism. Curr. Opin. Chem. Biol. 12, 25–31.
[49] Luheshi, L.M. et al. (2007) Systematic in vivo analysis of the intrinsic determinants of amyloid Beta pathogenicity. PLoS Biol. 5, e290.
[50] Iijima, K., Liu, H.P., Chiang, A.S., Hearn, S.A., Konsolaki, M. and Zhong, Y. (2004) Dissecting the pathological effects of human Abeta40 and Abeta42 in *Drosophila*: a potential model for Alzheimer's disease. Proc. Natl. Acad. Sci. USA 101, 6623–6628.
[51] Brignull, H.R., Morley, J.F., Garcia, S.M. and Morimoto, R.I. (2006) Modeling polyglutamine pathogenesis in *C. elegans*. Meth. Enzymol. 412, 256–282.
[52] van Ham, T.J., Thijssen, K.L., Breitling, R., Hofstra, R.M., Plasterk, R.H. and Nollen, E.A. (2008) *C. elegans* model identifies genetic modifiers of alpha-synuclein inclusion formation during aging. PLoS Genet. 4, e1000027.